



NITROGEN TRANSFORMATIONS ASSOCIATED WITH EARTHWORM CASTS

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Summary—Earthworms are intimately involved in the cycling of C and N in soil. Earthworm casts are enriched in mineral N; however, there have been few studies of the dynamics of microbial N transformations associated with earthworm casts. We evaluated the N-transformations in earthworm casts as affected by organic residues used as a food source by earthworms. Denitrification rate, nitrification potential and mineral N content of the casts of two earthworm species (*Octolasion tyrtaeum* Savigny and *Aporrectodea tuberculata* Eisen) were assessed in laboratory trials. Trials were made in plastic chambers (600 g soil) with three organic-C treatments: 20 g fresh hairy vetch (*Vicia villosa* Roth), 5.5 g air-dried hairy vetch or 5.5 g air-dried horse (*Equus caballus*) manure. Earthworm casts were enriched in mineral N, relative to surrounding soil, and that the amount of N accumulated in earthworm casts was a reflection of the N content of the organic matter used as a food source by the earthworms. Casts had elevated denitrification rates, compared to soil, however, rates were low relative to the elevated NO_3^- concentrations in the casts (80–100 $\mu\text{g NO}_3^- \cdot \text{N g}^{-1}$ dry wt). Observed denitrification rates appeared to be related to the quality of organic matter available to the earthworms, but were not significantly affected by species of worm.

INTRODUCTION

Earthworm casts have been found to contain elevated amounts of NH_4^+ , NO_3^- , Mg, K and P relative to surrounding soil (Lunt and Jacobson, 1944; Parle, 1963; Gupta and Sakal, 1967; Syers *et al.*, 1979; Syers and Springett, 1984; Tiwari *et al.*, 1989). Although earthworm casts are enriched in mineral N, there have been few direct studies of the microbially-mediated N transformations associated with earthworm castings.

Parle (1963) observed that freshly-deposited casts were high in NH_4^+ , but with time NH_4^+ decreased with a concomitant increase in NO_3^- , indicating high microbial nitrification activity. Similar findings were reported by Syers *et al.* (1979), who investigated the mineral-N dynamics of earthworm casts incubated for 12 days and found that 87% of the NH_4^+ initially present in casts was lost, but that increases in the NO_3^- pool did not match losses in NH_4^+ . It was suggested that the resulting N deficit (*ca* 25 $\mu\text{g-N g}^{-1}$) was due to a combination of immobilization and denitrification. In the few studies where denitrification rates of earthworm casts have been measured, high rates have been observed. Svensson *et al.* (1986), in controlled laboratory experiments, found that the casts of *Lumbricus terrestris* (L.) supported denitrification rates which were consistently higher than in surrounding soil. Surface casts collected from fertilized and unfertilized pasture plots also had enhanced denitrification activity (Elliott *et al.*, 1990).

Our objectives were to: (i) evaluate the effects of worm food source on microbial N transformations associated with earthworm castings, (ii) determine earthworm species effects on microbial N transformations, and (iii) assess the relative rates of denitrification associated with soil aggregates, worm castings and particulate organic matter in soil.

MATERIALS AND METHODS

Incubations

Laboratory trials were made by placing 600 g sieved (0.5 cm), air dried soil in plastic chambers. The soil was a Clarion loam (fine-loamy, mixed, mesic Typic Hapludolls) with a pH of 6.8. Treatments were prepared by mixing soil with either 20 g fresh hairy vetch (*Vicia villosa* Roth), 5.5 g air-dried hairy vetch or 5.5 g air-dried horse (*E. caballus*) manure. Both the fresh and dry hairy vetch were chopped (0.5 cm). Total C and N content of the soil, hairy vetch and horse manure amendments (Table 1) were determined by g.c. following combustion (Carlo Erba Instruments, Milano, Italy). Control pots receiving soil but no organic amendments were also prepared. The gravimetric water content of each treatment was adjusted to 35% by additions of distilled water, and treatments were conditioned overnight at 15°C. Each chamber was then inoculated with three mature individuals of *Oclolasion tyrtaeum* or *Aporrectodea tuberculata*. Both species are subsurface feeders; however, casts are deposited on the surface as well as

below the soil surface. Individuals of both species were obtained from colonies that were reared on partially-decomposed horse manure (Berry and Karlen, 1992). Three replicate pots of each treatment containing one species of earthworm were prepared and kept in the dark at 15°C. The pots were weighed every week and distilled water was added to maintain constant moisture conditions.

Sampling and analyses

Surface casts were collected from each pot once a week and denitrification rates of casts were measured. Two days prior to sampling the surface of each pot was cleared of all castings, thus all the earthworm casts examined in these experiments were ≤ 2 days old. Denitrification rates of casts was determined using the acetylene block method (Yoshinari *et al.*, 1977). Surface casts from each pot were placed in 10 ml test tubes (*ca* 0.5–2 g fr. wt), the tubes were stoppered (air headspace), and 1 ml C_2H_2 added to the headspace of each tube. Denitrification rates were calculated from N_2O production measured every 3 h over 12 h at 25°C. Nitrous Oxide was measured using a modification of an automated method (Parkin, 1985). At the final sampling (4 weeks) denitrification rates of particulate organic matter and soil aggregates were also determined. Identifiable pieces of particulate organic material, and soil aggregates (5–10 mm) were selected from each chamber, placed in 10 ml test tubes, and exposed to 10% C_2H_2 under aerobic conditions (air headspace). Nitrous oxide production rates were measured and denitrification rates expressed on a dry weight basis.

Cast samples were also analysed for NO_3^- and NH_4^+ . Casts were extracted with 2 M KCl (KCl:cast material = 5:1). Extracts were shaken for 2 h at 25°C, and then filtered. NO_3^- ($NO_3^- + NO_2^-$) was determined by the cadmium reduction method, and NH_4^+ determined by the indophenol blue method (Keeney and Nelson, 1982) using an automated chemistry unit (Lachat Instruments, Milwaukee, Wis.). NO_3^- and NH_4^+ results are expressed on a dry weight basis.

At the end of the experiment (4 weeks) the nitrification potential of casts and soil was also determined by the method of Schmidt and Belser (1982), modified as follows. Earthworm casts or soil samples from each pot (2–4 g fr. wt) were placed in flasks containing 25 ml of a 250 mM $(NH_4)_2SO_4$ solution. Flasks were stoppered with foam plugs and shaken mechanically at 25°C for 1 h. A 10 ml sample of the slurry

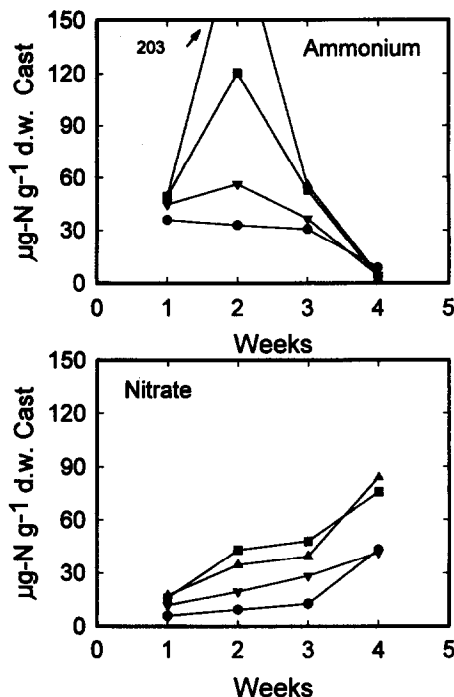


Fig. 1. NH_4^+ and NO_3^- concentrations of 2 day old casts of *O. tyrtaeum* at four times throughout the experiment. Symbols: no amendments (●), manure (▼), dry vetch (▲), green vetch (■).

was removed and analyzed for NO_3^- . The flasks were kept aerobic for 2 days, and then the slurries were filtered and NO_3^- determined. Nitrification potential was taken to be the difference in NO_3^- between the final and initial samplings. At each sampling duplicate soil cores (1 cm dia) were collected from each chamber, and analyzed for NO_3^- and NH_4^+ . Sub-samples of casts and soil were also collected for determination of water content. Rates of nitrification are expressed on a dry weight basis.

RESULTS AND DISCUSSION

Nitrate and ammonium dynamics

After 1 week of incubation NH_4^+ concentrations of the casts of *O. tyrtaeum* were not significantly affected by food source (Fig. 1); however, at 2 weeks a peak of NH_4^+ was observed in the castings of *O. tyrtaeum* fed on green or dry hairy vetch. Significantly higher NH_4^+ concentrations were observed in the treatments with dry vetch as compared to green vetch. This result may reflect a feeding preference of *O. tyrtaeum* for dry rather than green vetch. (Bostrom (1987) reported that *Allolobophora caliginosa* was sensitive to fresh lucerne (*Medicago sativa* L.), and suggested that toxic substances released during decomposition were responsible.

While there was a trend for the casts of worms fed manure to contain more NH_4^+ after 2 weeks, these concentrations were not significantly different from the control pots which received no added organic

Table 1. C and N content of soil and organic residues

Material	C	N
	g kg ⁻¹	
Soil	20.2 (0.47)	1.44 (0.085)
Hairy vetch	407.0 (3.16)	43.1 (1.51)
Manure	273.0 (14.0)	18.8 (0.72)

All materials were air dried before analyses. Means are determinations of six samples. Numbers in parentheses are standard deviation.

residues. After 2 weeks NH_4^+ concentrations in the castings of all treatments decreased. The decrease in NH_4^+ concentrations of the casts was mirrored by increases in NO_3^- concentrations (Fig. 1). Throughout the experiment NO_3^- concentrations of the castings tended to be greater in the hairy vetch treatments than in the pots with manure or no added organic residues.

The temporal trends of NH_4^+ and NO_3^- associated with the casts of *A. tuberculata* (Fig. 2) were similar to those exhibited by *O. tyrtaeum*. In all of the organic residue treatments, maximum NH_4^+ concentrations were observed in the castings harvested at 2 weeks. Concentrations of NH_4^+ had decreased in casts harvested at later times. As observed with the *O. tyrtaeum* castings, NO_3^- concentrations associated with casts from *A. tuberculata* increased throughout the experiment. Highest NO_3^- concentrations were observed in incubations with green vetch. There was a trend of higher NO_3^- concentrations associated with the dry vetch and manure treatments; however, concentrations were not significantly different than castings from worms incubated with no added organic residue.

To assess the effects of earthworms on N transformations, NH_4^+ and NO_3^- concentrations were measured in pots amended with the different organic residue treatments in the absence of earthworms (Fig. 3). The peak of NH_4^+ concentrations in the worm castings observed at 2 weeks did not occur in soil incubated without earthworms. In the absence of

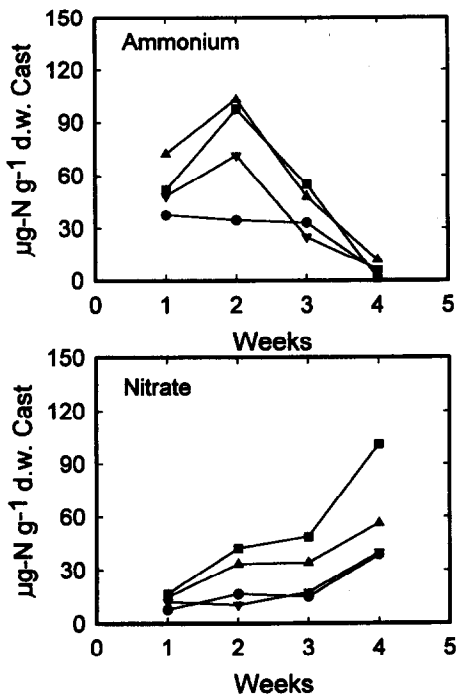


Fig. 2. NH_4^+ and NO_3^- concentrations in 2 day old castings of *A. tuberculata* at four times throughout the experiment. Symbols: no amendments (●), manure (▼), dry vetch (▲), green vetch (■).

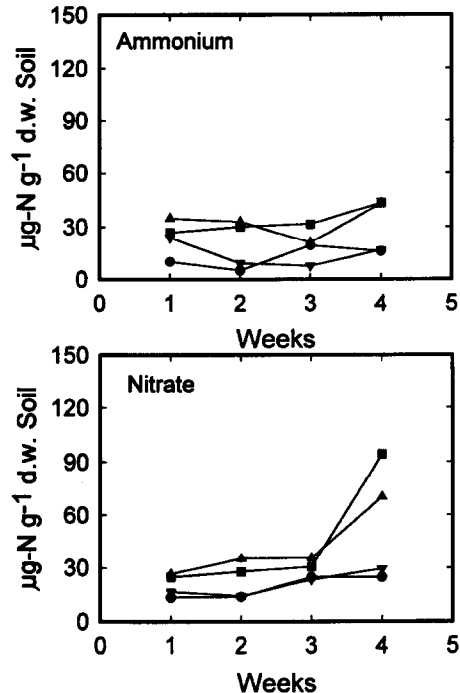


Fig. 3. NH_4^+ and NO_3^- concentrations in soils with no amendments (●), manure (▼), dry vetch (▲), or green vetch (■).

earthworms, soil NH_4^+ concentrations were relatively constant in the range of 5–20 $\text{mg NH}_4^+-\text{N g}^{-1}$ in the control and manure treatments, and 26–45 $\text{mg NH}_4^+-\text{N g}^{-1}$ in the vetch amended pots.

The pattern and concentrations of soil NO_3^- in pots without worms were very similar to that observed in the earthworm casts. Soil NO_3^- in pots without earthworms exhibited a general trend of increasing concentration with prolonged incubation (Fig. 3). Only slight increases in NO_3^- were observed in pots with manure or no organic amendment; however, considerable increases in NO_3^- were observed at 4 weeks in the hairy vetch amended pots, with concentrations exceeding 60 $\mu\text{g-N g}^{-1}$.

Denitrification

Within a given treatment, and for a given earthworm species, denitrification rates were not significantly different over the four sampling events, so the data for each sampling were pooled, and differences as a function of organic residue treatment evaluated (Table 2). Denitrification rates associated with the casts of either worm species followed the same general trend. Denitrification activity associated with casts produced from worms in soil with no organic amendments were low. Although denitrification in casts from the manure treatment tended to be higher, they were not significantly different from treatments without added organic residue. Castings from worms feeding on hairy vetch exhibited higher denitrification rates than castings from worms fed manure or no added organic material. Denitrification rates of

O. tyrtaeum casts generated in the dry vetch treatment were significantly higher than the control, and *A. tuberculata* casts produced in both vetch treatments had significantly higher denitrification rates than casts in the manure or control treatments.

Past research indicates that earthworm castings have high specific denitrification rates relative to the bulk soil. Denitrification rates of earthworm casts collected from experimental pasture plots were reported by Elliott *et al.* (1990) to be 2–3-fold greater than the surrounding soil. Specific denitrification rates of casts ranged from 130 to 410 ng N g⁻¹ d⁻¹ for 0 N fertility plots, and 110 to 1600 ng N g⁻¹ d⁻¹ for plots fertilized at 200 kg N (Elliott *et al.*, 1990). In such a field study, it was impossible to determine the specific worm species or the source of organic material used by the earthworms. In laboratory studies, Svensson *et al.* (1986) found enhanced denitrification rates associated with the casts of *Lumbricus terrestris* fed on lucerne (*Medicago sativa* L.), an N-rich material. The average denitrification rate of 2 day old casts computed from their study (360 ng N g⁻¹ d⁻¹) is in the range of denitrification rates we report here.

High denitrification activity is associated with particulate organic matter in structureless soils (Parkin, 1987; Christensen *et al.*, 1990) or with stable soil aggregates in structured soils (Sexstone *et al.*, 1985; Seech and Beauchamp, 1988; Smith, 1990; Arah, 1990). Accordingly, we compared the denitrification rates associated with earthworm castings with rates associated with other potential denitrifying microsites in soil. Specific denitrification rates and moisture contents of individual soil aggregates (2–10 mm dia), individual pieces of particulate organic material (manure, green vetch and dry vetch), and casts of earthworms fed different food sources are presented in Table 3. The highest denitrification rates were those associated with green vetch. Denitrification rates of the dry vetch and the casts of earthworms fed on hairy vetch were not significantly different and exhibited rates in the range of 200–250 ng-N denitrified g⁻¹ d⁻¹. Denitrification rates of casts of earthworm fed on manure or soil organic matter were lower and not significantly different from the specific denitrification rates of individual pieces of manure or soil

Table 3. Specific denitrification rates and water content of soil aggregates, worm castings and different types of particulate organic matter

Microsite	Denitrification rate	Water content	n
	— ng-N g ⁻¹ d ⁻¹ —	— g g ⁻¹ dry wt —	
Soil aggregate	4.7a (7.29)	0.334a (0.024)	27
Manure	21.5a (26.0)	1.458b (0.316)	9
Green vetch	2470.0b (2450)	1.304b (0.408)	9
Dry vetch	242.0c (508)	1.104b (0.456)	9
Castings (vetch fed)	209.0c (219)	0.341a (0.029)	30
Castings (manure/soil o.m. fed)	16.9a (9.89)	0.348a (0.050)	36

Values within a column followed by different letters are significantly different ($P \leq 0.05$).

Values in parentheses are 1 SD.

aggregates. The lack of differences in water content between casts and soil indicate that differences in denitrification were not due to moisture. Rather, the denitrification associated with earthworm casts reflected denitrification rates associated with the organic C amendment used as a food source by the earthworms.

Nitrification

Nitrification activity of earthworm casts was assessed at 4 weeks (Table 4). No differences in nitrification activity as a function of earthworm species were observed; however, food source did have a significant effect. The casts of earthworms fed horse manure or soil organic matter supported lower nitrification activity than casts of worms fed hairy vetch. Nitrification activity of the soil was also affected by organic residue addition. Soil with no amendments or with added horse manure had lower nitrification potentials than soil in which hairy vetch was added, although, for any given organic matter treatment, nitrification rates of the soils were approximately one-tenth of the activity associated with worm castings. Although, Svensson *et al.* (1986) observed that production of N₂O from nitrification was insignificant, other studies indicate that earthworm casts have high associated nitrification activity (Parle, 1963; Syers *et al.*, 1979). Our results confirm earlier reports of high nitrification potential associated with earthworm casts, and in addition, indicate that nitrification potential reflects the N content of the organic materials used as a food source by earthworms.

Table 2. Effect of food source on denitrification rates of castings of *O. tyrtaeum* and *A. tuberculata*

Added food source	Mean denitrification rate	
	<i>O. tyrtaeum</i>	<i>A. tuberculata</i>
	— ng-N g ⁻¹ d ⁻¹ —	
None	6.7a (180)	5.6a (124)
Manure	27.1a (133)	7.8a (152)
Green vetch	113.0ab (211)	232.0b (81.9)
Dry vetch	385.0b (183)	217.0b (116)

Values within a column followed by different letters are significantly different ($P \leq 0.05$) as determined by Gabriel's multiple comparisons test.

Values in parentheses are % coefficient of variation.

Table 4. Nitrification activity associated with soil and castings produced by earthworms incubated with different organic carbon food sources

Added food source	Mean nitrification potential		
	<i>O. tyrtaeum</i>	<i>A. tuberculata</i>	Soil
	— µg-N g ⁻¹ d ⁻¹ —		
None	28.9a (37.9)	41.0a (59.0)	4.9a (16.7)
Manure	24.7a (27.4)	35.3a (34.0)	9.8a (28.9)
Green vetch	89.6ab (49.3)	117.2b (34.8)	18.9b (15.7)
Dry vetch	126.0b (51.7)	122.7b (25.6)	16.3b (17.7)

Values within a column followed by different letters are significantly different ($P \leq 0.05$).

Values in parentheses are % coefficient of variation.

Conclusions

It is difficult, from our laboratory experiments, to develop quantitative extrapolations concerning the effects of earthworms on transformations of organic-N in soil. This is, primarily, because the cast deposition rate is not known. In our study only surface casts were collected, yet both species are known to also deposit casts within the soil. However, qualitatively, the following conclusions can be drawn: first, our studies confirm earlier observations that earthworm casts are enriched in mineral N. In addition, our study indicates that the magnitude of N accumulation in earthworm casts reflects the N content of the organic matter used as a food source by the earthworms. This supports observations of Syers *et al.* (1979) who suggest that earthworms are relatively inefficient at extracting N from organic materials. While earthworms may be inefficient at extracting N from organic residues, the elevated cast- NH_4^+ concentrations do indicate that earthworms accelerate N-mineralization from organic residues. There appeared to be no species effect on N accumulation in the casts of earthworms, although the NH_4^+ concentrations in the casts of *O. tyrtaeum* suggest a possible feeding preference of this species for dry rather than fresh vetch. *O. tyrtaeum* is primarily a geophagous species and feeds primarily on roots and other organic materials beneath the soil surface.

In soil devoid of earthworms there was no transient accumulation of NH_4^+ , and NO_3^- concentrations of 2 day old casts increased during the 4 week experiment. A transient accumulation of NH_4^+ was observed in the earthworm casts. This suggests that, in the absence of earthworms, the rate-limiting step in the formation of NO_3^- was the decomposition rate of the organic matter, whereas in the presence of earthworms, the mineralization rates of organic matter was increased and nitrification was limiting the rate of NO_3^- production. Again, our data support Syer *et al.*, who suggest that the primary effect of earthworms on N-cycling in soil is to increase the rate of organic-N mineralization.

We observed elevated denitrification rates associated with earthworm casts as compared to soil. Denitrification rates of casts did not appear to be influenced by species of worm; however, denitrification was affected by the food source available to the earthworms. In nature different species of earthworms may indeed exhibit feeding preferences, and our results suggest that the denitrification rates of the casts would be a reflection of the 'quality' of organic C ingested. Highest denitrification rates were associated with vetch-fed worms, and lowest denitrification rates were associated with casts from worms feeding on the partially-decomposed plant residue resident in manure or native soil organic materials. Maximum rates of denitrification associated with vetch-fed earthworms were on the order of $0.2 \mu\text{g NO}_3^- \text{-N denitrified g cast}^{-1} \text{d}^{-1}$. These rates are relatively

low compared to NO_3^- concentrations in the casts ($80\text{--}100 \mu\text{g NO}_3^- \text{-N g}^{-1}$). Thus, even though worm casts provide a favorable microsite for denitrification, the increased NO_3^- pools observed in the casts are not markedly affected over the short term. It was also observed that, although higher denitrification was observed in earthworm casts, these rates were lower than those associated with isolated pieces of particulate organic material which had not previously been ingested by earthworms.

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